Spatial regulation of mTORC1 signalling: beyond the Rag GTPases

Bernadette Carroll[1]

[1]School of Biochemistry, Biomedical Sciences Building, University Walk, Bristol, BS8
email: bernadette.carroll@bristol.ac.uk

Abstract

The mechanistic (or mammalian) Target of Rapamycin Complex 1 (mTORC1) is a central regulator of cell growth and metabolism. By integrating mitogenic signals, mTORC1-dependent phosphorylation of substrates dictates the balance between anabolic, pro-growth and catabolic, recycling processes in the cell. The discovery that amino acids activate mTORC1 by promoting its translocation to the lysosome was a fundamental advance in the understanding of mTORC1 signalling. It has since become clear that the lysosome-cytoplasm shuttling of mTORC1 represents just one layer of spatial control of this signalling pathway. This review will focus on exploring the subcellular localisation of mTORC1 and its regulators to multiple sites within the cell. We will discuss how these spatially distinct regions such as endoplasmic reticulum, plasma membrane and the endosomal pathway co-operate to transduce nutrient availability to mTORC1, allowing for tight control of cell growth.

Keywords: Rheb, autophagy, lysosome, trafficking, endosome, amino acids, endoplasmic reticulum, Golgi

1. Introduction:

The mechanistic target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that forms the catalytic subunit of two spatially, structurally and functionally distinct complexes. mTOR in complex with Raptor, mLst8, DEPTOR and PRAS40 constitutes mTOR complex 1 (mTORC1), while mTOR complex 2 (mTORC2) is composed of mLst8, Rictor and mSin1. mTOR was first identified over two decades ago, and mTORC1 and mTORC2 have since been established as fundamental regulators of cell growth and metabolism[1-3]. The two mTOR complexes integrate some common activators such as growth factor-dependent phosphoinositide 3-kinase (PI3K) signalling to phosphorylate distinct downstream targets. The recruitment of these substrates is dictated by the differential scaffolding components of the complexes, i.e. Raptor vs Rictor[2, 3]. Broadly speaking, the major role of mTORC1 is to integrate the availability not only of growth factors, but also amino acids, oxygen and energy to promote biosynthesis of proteins, lipids and nucleotides. One of the key functions of mTORC1 is to drive protein translation via phosphorylation of proteins such as p70S6K and the ribosome-associated 4EBP1 and by promoting ribosome biogenesis[2, 3]. The other major role for

mTORC1 is to inhibit the catabolic process of macroautophagy, hereafter referred to as autophagy, a cellular recycling process that can sequester contents of the cytoplasm either in bulk or selectively into specialised organelles called autophagosomes. Mature autophagosomes are trafficked along microtubules and ultimately fuse with lysosomes to form hybrid, degradative compartments called autolysosomes. The contents are then degraded and macromolecules such as amino acids and fatty acids can be liberated into the cytoplasm to support cellular growth and metabolism. Autophagy occurs in all cell types at basal levels that vary depending on cell function and metabolic demands but it is rapidly upregulated upon inhibition of mTORC1[4, 5]. mTORC1 regulates autophagy via two major routes, firstly phosphorylation of ULK1/Atg1 kinase which is essential for autophagy induction and secondly by phosphorylating members of the TFEB family of transcription factors[4-6]. TFEB is a master regulator of autophagosome and lysosome gene transcription which is restrained in the cytoplasm by mTORC1 phosphorylation[6]. The second complex, mTORC2 on the other hand is implicated in controlling the cytoskeleton and membrane tension, proliferation and cell survival via substrates including Akt, PKC and SGK1[3, 7].

In this review we will explore how mTOR, in particular mTORC1, is controlled by changes in the subcellular localisation of the complex itself and its regulators. The nutrient-dependent shuttling of mTORC1 between the cytoplasm and the late endosome/lysosome compartment, a discovery that fundamentally advanced our understanding of this signalling pathway has been the focus of several excellent reviews elsewhere[1-3, 8, 9]. Therefore, here we predominantly focus on other aspects of mTORC1 regulation by its spatial organisation within the cell. In particular, we will explore how increasing evidence places key mTORC1 regulators at multiple different sites in the cell including endoplasmic reticulum and plasma membrane. We will discuss how the endosomal and lysosomal pathways influence mTORC1 signalling and integrate these new insights to describe the emerging spatially dynamic model of mTORC1 activation.

2.1 mTORC1 at the lysosome

In the presence of amino acids, mTORC1 localises to vesicular structures in mammalian cells that are positive for the late endosomal marker, Rab7 and lysosomal marker, Lamp1[10]. This may indicate that mTORC1 localises to hybrid endolysosomal structures which contain active acid hydrolases to degrade cargo, as opposed to mature storage granule-like lysosomes [11]. However, since localisation of the mTORC1 substrate, TFEB has been observed on both endolysosomes and lysosomes, it indicates the functional mTORC1 complex is likely present on both structures [11]. TORC1 localisation to the vacuole membrane (equivalent of the lysosome) is conserved in yeast, indicating that this localisation is an important feature of

mTORC1 signalling, forcible removal of mTORC1 from this site inhibits its activity[3, 9]. mTORC1 recruitment and retention on the lysosome is dependent on the atypical Rag family of Ras-related small GTPases. There are four Rag proteins in mammals where RagA and RagB are functionally redundant and dimerise with either RagC or RagD, while in yeast the functional complex consists of Gtr1 and Gtr2 (homologues of RagA and RagC).

Identifying the molecular mechanisms controlling the Rag-mTORC1 axis is a very active and dynamic area of research. Here we will describe the current model briefly as the focus of this review to explore the spatial control of mTORC1 more broadly. mTORC1 predominantly senses three amino acids, leucine, arginine and glutamine via a signalling cascade that leads to the GTP-loading of RagA/B and hydrolysis (of GTP to GDP) of RagC/D to form a heterodimer that can directly interact with mTORC1[9]. Cytoplasmic sensors for leucine and arginine, Sestrin2 and CASTOR1/2 respectively, signal via a complex called GATOR2 to inhibit the RagA GTPase-activating protein (GAP) complex, GATOR1 so that in the presence of amino acids, RagA is in GTP-bound form, promoting a direct interaction with mTORC1, likely in the cytoplasm[9, 12]. The Rag-mTORC1 complex is then recruited to the lysosomal surface via the multi-protein RAGulator complex consisting of p18 (also known as LAMTOR1), p14 (LAMTOR2), MP1 (LAMTOR3), LAMTOR4 and LAMTOR5 [8, 9].

Several lysosomal transmembrane proteins co-operate with Rag-Ragulator axis to form a large, dynamic complex that tightly controls mTORC1 recruitment, retention and release from the lysosome. Specifically, the vacuolar ATPase (V-ATPase) proton pump which controls lysosomal pH, the amino acid transporter, SLC38A9 and the cholesterol transporter, NPC1 have all been specifically implicated in transducing the availability of amino acids (particularly arginine) or cholesterol to mTORC1 from within the lysosomal lumen, in a Rag-dependent manner[1, 8, 9]. See **Figure 1** for a diagram of the lysosome-localised mTORC1 machinery. The mechanistic details of how the availability of amino acids and other mitogens inside the lysosome versus in the cytoplasm is integrated by Ragulator-Rag are yet to be fully realised but for example conformational changes in V-ATPase and SLC38A9 in the presence of amino acids are required for mTORC1 activation [9, 13-15].

A wide range of additional mechanisms have been implicated in relaying the availability of free amino acids, including glutamine (via it's metabolism to α-ketoglutarate[16]; and Arf1 activity [17]), asparagine (via Arf1)[18] and leucine [19], phospholipase D[20, 21] and energy [22] to mTORC1 via control of protein localisation to the lysosomal surface. These are reviewed elsewhere [3, 9, 23]. Growth factor and energy availability is primarily transduced to mTORC1 via nucleotide loading of the small GTPase Rheb. Signalling downstream of PI3K/Akt, MEK/Erk and AMPK pathways leads to phosphorylation and inactivation of the Rheb-GAP, tuberous sclerosis

complex (TSC), which consists of three subunits, TSC1, TSC2 and TBC1D7. Amino acids, in particular arginine, cooperate with growth factors to ensure tight control of TSC localisation and thus activity[24, 25] (**Figure 1**).

The canonical model of mTORC1 activity works on the premise that mTORC1 is recruited to the lysosomal surface to facilitate its direct interaction with the small GTPase, Rheb in its GTP-bound state, leading to a conformational change that activates mTORC1 by allowing access of substrates to the kinase domain. Indeed, there is ample evidence to support this hypothesis, including the observation that Rheb (both endogenous and overexpressed) is localised to the lysosome [10, 26-30] and that starvation-induced recruitment of TSC to the lysosome is Rheb-dependent [24, 26]. Rheb has however been equally noted to localise to a wide range of other endomembranes [27, 31, 32 33]and we will discuss the repercussions of this for mTORC1 in section 2.3.

While overexpression of dominant-negative Rag GTPases, and forcible removal of mTORC1 from the lysosome inhibits mTORC1, its global redistribution from the lysosome is not an absolute requirement to inactivate mTORC1. There are multiple reported cases whereby mTORC1 activity can still be inhibited but is retained on the lysosomal membrane, including in TSC2-null cells [25] and indeed there is still a significant proportion of mTOR-Lamp1 co-localisation in starved HeLa cells (~30%, down from 50-60%) [24, 26]. This implies that conformational changes within the large lysosome-localised protein complex are sufficient to either preclude the mTORC1-Rheb interaction or mTORC1-substrate interactions.

2.2 Why the lysosome?

It is becoming increasingly clear that the lysosome is not simply a platform to support mTORC1-associated protein complexes but is rather an active participant in signalling and the lysosome plays a central role in coupling cellular anabolism and catabolism[8, 23, 34]. For example, loss of lysosomal membrane integrity inhibits mTORC1; damaged lysosomes leak luminal galactosides that are recognised by galectin8, the recruitment of which inhibits Rag-dependent mTORC1 activity[35]. Furthermore, inhibition of lysosomal function, via targeting protease activity or the V-ATPase (which increases pH and reduces the activity of lysosomal hydrolases) can inactivate mTORC1 and activate autophagy [13, 36]). Some of these effects can be attributed to a direct, although poorly understood role for the V-ATPase in controlling mTORC1 activity (see [9, 13]). However, one reason why the lysosome is a prime site for mTORC1 residence is that lysosomal degradation generates free amino acids that can directly and indirectly activate mTORC1 (**Figure 1**). For example, mTORC1 reactivation after prolonged periods of starvation is dependent on liberation of amino acids via the autophagy-lysosome pathway [37, 38]. This reactivation of mTORC1 acts to reduce autophagy levels, limiting

degradation and promoting cell survival. Indeed, there is evidence that amino acid generation via the autophagy-lysosome pathway can be exploited to support ectopic activity of mTORC1 in multiple models including cellular senescence[39]. Interestingly, while bulk autophagy would presumably replenish the entire intracellular pool of amino acids at ratios detected in the proteome, lysosome-dependent mechanisms have also been described that generate specific amino acids, glutamine [38] and arginine [40]. The selective autophagic degradation of ribosomes can specifically help replenish arginine levels to activate mTORC1 while the generation of glutamine and its subsequent conversion to glutamate replenishes the pools of non-essential amino acids (NEAA) to activate mTORC1 during autophagy. Intriguingly, autophagy does not appear to contribute to the replenishment of essential amino acids (EAA) in myotubules [38, 41], potentially because EAA are very rapidly utilised. Indeed, starvation has widely been shown to cause a significant reduction in EAA whole cell extracts [38, 39, 42], however fascinatingly, their levels are maintained within lysosomes, via a mechanism that involves SLC38A9[42]. By spatially segregating EAA into lysosomes, it may protect them from depletion via metabolism or protein translation during starvation [42].

The spatial relationship between free amino acids and mTORC1 activation remains an evolving area of research. As discussed above, there are cytoplasmic sensors for the most potent of mTORC1 activators, leucine and arginine while there are complex mechanisms via which glutamine appears to be transduced to mTORC1 including via metabolism (to α-ketoglutarate)[16] and the Golgi-localised small GTPase, Arf1 [17, 43]. Most recently, sensing of asparagine was also found to occur via an Arf1-dependent mechanism[18]. These amino acids can be taken up from the environment by membrane transporters[44, 45] or via macropinocytosis (as polypeptides)[46, 47], they can be effluxed from the lysosome into the cytoplasm [48] and/or sensed from within the lysosome [13, 49]. The 'inside-out' mechanisms of amino acid sensing involves free amino acids in the lysosomal lumen binding and causing a conformational change in V-ATPase[13], while arginine-binding to SLC38A9 enhances its transport of polar amino acids to the cytoplasm where they can activate amino acid sensors [15, 49]. Overexpression of the amino acid transporter, PAT1, has been shown to both activate mTORC1 (by replenishing the local, cytoplasmic concentrations of amino acids)[48] and inactivate mTORC1 (by depleting the lysosome of amino acids) [13]. The multiple routes via which amino acid availability (**Figure 1**) can be transduced to mTORC1 demonstrates the exquisite level of spatial and temporal control of mTORC1 signalling. The further development of techniques to measure individual amino acids and metabolites from isolated lysosomes and potentially other organelles such as mitochondria, will undoubtedly improve our resolution and understanding of the spatial relationship between amino acids, their metabolism and mTORC1 activity. This will path the way to our understanding of whether different amino acids are

sensed preferentially in the cytoplasm versus lysosome, and whether different sites of sensing can illicit different downstream effects.

2.3 Where is Rheb?

The localisation of the activating small GTPase, Rheb remains one of the most elusive pieces of the mTORC1 signalling puzzle. Rheb binds directly to mTORC1, causing a conformational change that exposes the kinase domain to substrates[50, 51]. The consensus model of mTORC1 signalling places Rheb on the lysosomal membrane [10, 26-30] creating a logical rationale that the Rag/Ragulator axis has evolved to support mTORC1 recruitment to this same membrane compartment and facilitate direct interaction between mTORC1-Rheb. However, while there are a significant number of reports of Rheb localising to lysosomes, numerous other reports place Rheb on ER membranes, Golgi membranes [27, 31, 32] and peroxisomes [33] (**Figure 2**).

Recent insights into the biology of Rheb could bring together these previously conflicting data. Small GTPases contain a C-terminal CAAX box which is post-translationally modified with the addition of a farnesyl group to support recruitment of the proteins to endomembranes. Upstream of this is a hypervariable region with secondary targeting sequences. For example, in addition to farnesylation, Ras is also palmitoylated which promotes its localisation to the plasma membrane. Rheb however appears to lack any specific secondary targeting sequence in the hypervariable region and a recent report suggests that rather than being specifically anchored and enriched on lysosomes, Rheb can target endomembranes indiscriminately and that transient localisation of Rheb to lysosomes is sufficient to activate mTORC1[32]. Since many reports place Rheb on ER and Golgi membranes, one hypothesis has been these Rheb-positive membranes interact with mTORC1-positive lysosomes and these contact sites facilitate mTORC1 activation [31]. Constitutive targeting of Rheb to the ER membrane however fails to activate mTORC1 despite the fact that lysosome-ER contacts are still observed, indicating that Rheb does not activate mTORC1 by bridging across the two membranes[32]. Rather, the authors suggest that the ER localisation often identified for Rheb is simply a default localisation of farnesylated proteins[32].

Without a specific targeting sequence, how exactly is the dynamic flux of Rheb between membranes and the cytoplasm controlled? Recently, a mechanism of spatial cycling of Rheb was described which acts to deliver and enrich Rheb specifically on lysosomes, despite the absence of a dedicated targeting sequence [30]. The model proposes that the interaction between Rheb and a GDI-like solubilising factor (GSF), PDEδ [52] maintains Rheb-GDP in the cytoplasm. Soluble Rheb has previously been shown to have increased nucleotide exchange compared to membrane-bound Rheb [53, 54] and thus the authors suggest that PDEδ supports Rheb nucleotide exchange (GTP loading) in the cytoplasm. If this is correct, it could negate a

need for, or argue against the existence of a Rheb-GEF, a long postulated but elusive regulator of mTORC1 signalling to maintain the very high levels of Rheb-GTP observed in the cell [55]. Rheb-GTP is released from PDEδ upon PDEδ-Arl2-GTP binding, an interaction that occurs predominately in the perinuclear region (**Figure 1**). The authors suggest this interaction locally enriches Rheb-GTP in proximity to lysosomes thus favouring its recruitment to this site[30]. There are conflicting views indicating that the interaction between Rheb and mTORC1 then supports retention of Rheb on lysosomes [30] while another report suggests that Rheb-GTP cooperates with Rag GTPases to recruit or retain mTORC1 on lysosomal surfaces[25]. Specifically, overexpression of active Rheb was found to be sufficient to recruit mTORC1 to the lysosomal membrane even in the absence of amino acids and indeed in *TSC2*$^{-/-}$ MEFs, where Rheb is constitutively active, mTORC1 fails to redistribute to the cytoplasm upon starvation [25].

2.4 Rheb-TSC interaction

The spatial cycling model for Rheb regulation is completed by TSC-dependent hydrolysis of Rheb, promoting its re-solubilisation via PDEδ[30]. Inhibition of TSC by genetic KO or insulin, leads to increased Rheb-GTP on membranes[30] while activation of TSC during starvation, leads to its recruitment to lysosomal membranes via a poorly understood mechanism that has been postulated to be Rheb-dependent and Rag GTPase-dependent [24-26]. Our own data indicate that overexpression of wild-type or active Rheb is sufficient to recruit TSC to the lysosome [24]. We have hypothesised that TSC recruitment to the lysosome is able to physically limit the interaction between mTORC1 and Rheb; TSC can prevent constitutively active Rheb-dependent mTORC1 signalling while over-expression of TSC2 GAP-dead mutants are also still able to inhibit mTORC1 activity. There is still a lot to understand regarding the complex spatial relationship between TSC-Rheb-mTOR at the lysosome; for example, does Rheb recruit mTORC1? Does mTORC1 help retain Rheb on lysosomes? and how or why is TSC stably recruited to the lysosomal membranes in starvation, especially if starvation leads to a reduction in Rheb from the lysosomal membrane? And does the TSC complex indeed have additional roles in controlling the Rheb-mTORC1 axis than simply acting as a Rheb-GAP? Furthermore, TSC and Rheb have also been observed to localise to peroxisomes to control mTORC1 in response to oxidative stress[33], so could different stress/nutrient mechanisms control the site-specific localisation of TSC or Rheb?

2.2 Lysosomal localisation

As we have discussed, lysosomal function and membrane integrity are essential for proper control of mTORC1. Often however we depict lysosomes as discrete, terminal degradative units while in reality they are extremely dynamic organelles that are capable of moving at 0.2-

0.6μm/s^{-1} [56, 57], and are in constant flux with trafficking pathways delivering intracellular and extracellular cargo for degradation via the autophagy and endocytic/macropinocytic pathways, respectively. Importantly, the subcellular distribution of lysosomes is dictated by mitogenic signals and correlates with mTORC1 activity[34, 58-60]. In particular, addition of the growth factor-rich supplement foetal calf serum (FCS) to culture media and the resulting reduction in intracellular pH leads to kinesin-dependent dispersal of lysosomes along microtubules, to the cell periphery [58, 61-63]. Nutrient starvation on the other hand promotes the dynein-dependent clustering of lysosomes in the juxta-nuclear region, a localisation that supports starvation-induced autophagy via mTORC1 inhibition [58], and by facilitating increased activity of lysosomes (i.e., lower pH and increased access to Golgi-derived newly synthesised lysosomal hydrolases compared to lysosomes at the periphery) [64] (**Figure 1 and 2**).

Peripheral localisation of mTORC1-positive lysosomes directly contributes to mTORC1 activity and is proposed to be facilitated by proximity to mitogenic inputs i.e. growth factor receptors, amino acid receptors, macropinosomes. Indeed an enrichment of phosphorylated Akt at the plasma membrane is observed following serum addition [58, 59] and ~70% of free cytoplasmic leucine is taken up via plasma membrane transporters such as LAT1 (SLC7A5)[45]. Forced peripheral spreading of lysosomes by overexpression of the small GTPase ARL8B, which couples lysosomes to kinesins, is sufficient to increase mTORC1 activity, albeit marginally, but this forced re-localisation is neither sufficient for mTORC1 activation in starvation nor is it absolutely necessary [58, 60]. For example, lysosomal clustering by ARL8B loss, knock-out of kinesin proteins, KIF1B-KIF5B or the BORC component, myrlysin (BORC is a lysosome-localised multi-subunit complex that facilitates ARL8B recruitment) delays but does not inhibit the reactivation of mTORC1 following starvation and the effect may be specific to serum but not amino acids [58, 60]. In another study however, amino acids have been implicated in lysosome spreading and thus mTORC1 activation. In this model, amino acid replenishment activates the Vps34-dependent production of PI3P on lysosomes, promoting the PI3P and Rab7-dependent interaction of lysosomes with the ER-resident protein protrudin1 which facilities the transfer of kinesins to the PI3P-binding protein FYCO1 [59, 65]. Amino acid starvation on the other hand promotes recruitment of the RagC GAP, Folliculin to the lysosome which supports clustering of lysosomes by tethering them to the Golgi via Rab34-RILP complex[66]. Therefore, while the role of amino acids in controlling mTORC1 activity is clear, their role in controlling lysosome spreading remains to be clarified.

Common regulators couple lysosome localisation and mTORC1 activation, adding further layers of co-regulation between the two. Specifically, the Ragulator component p18/LAMTOR1 interacts with the BORC complex [67], an association that is reduced in the presence of EGF [68]. Reduced BORC-p18 binding leads to increased BORC-dependent recruitment of ARL8B to

late endosomes/lysosomes to promote their dispersal to the periphery. Thus, p18 plays a dual role, regulating the recruitment of mTORC1 to the lysosome and the movement of mTORC1-positive lysosomes to a region enriched in mitogens.

In addition to mTORC1, at least a portion of mTORC2 also reside on endosomes [69] and lysosomes and their localisation also controls growth factor activation of mTORC2 substrates [60]. Further pools of mTORC2 have been noted at the plasma membrane, mediated by PH-domain containing subunit mSin1 and the adaptor, syndecan 4 [69-71]. Further work to explore the spatial and temporal control of mTORC1 versus mTORC2 on lysosomal membranes, how mTORC2 is recruited to lysosomal membranes and what, if any the specific role for this pool of mTORC2 will expand our knowledge of lysosome-controlled cellular metabolism.

The peripheral localisation of lysosomes thus spatially integrates nutrient input with mTORC1 activation and in the next section we will be explore how together they may co-operate to a previously unrecognised degree at the cell surface to control cell size, growth and migration.

2.5 mTORC1 and the plasma membrane

Although the increased activation of mTORC1 in cells with peripheral lysosomes has been postulated to be as a result of proximity to mitogenic inputs, it's not clear whether this occurs in any specific region of the plasma membrane, is facilitated by direct interactions or via signal transduction and whether there is any site-specific role for mTORC1 activity at the cell periphery (**Figure 1**).

Peripheral lysosomes contribute to the turnover of focal adhesions, integrin-based complexes that anchor cells to the extracellular matrix. Specifically, the Ragulator components, p14-MP1/LAMTOR2-3 are required to remove IQGAP from focal adhesions to promote cell migration [72]. The mTORC1-inhibitor, rapamycin also inhibits cell migration via a mechanism involving S6K and 4E-BP1[73], raising the possibility that mTORC1 on lysosomes in the periphery may co-operate to facilitate a site-specific role in the turnover, maintenance or stability of adhesion complexes either via tight, localised regulation of protein translation or autophagy-lysosome dependent degradation. This concept would be particularly interesting to explore in cancer cells that are frequently characterised by increased mTORC1 activity and often display a peripheral distribution of lysosomes [74, 75]. At the same time a recent report demonstrated that increased stiffness of the extracellular matrix can increase mTORC1 activity via cooperation with integrins in breast cancer[76]. Alternatively, lysosomal pools of mTORC2 [69] could regulate the cytoskeleton [77] at the periphery to control cell migration. Further studies are required to determine if there is a functional link required for cancer cell growth, migration, metastases or survival.

Furthermore, although there is currently no known role identified for mTORC1, BORC-dependent re-localisation of lysosomes to the cell periphery and their subsequent exocytosis provides membrane to support plasma membrane repair and increased cell size [67, 78-80]. So, while there are several reported roles for lysosomes in the cell periphery, there has been little focus on whether mTORC1, localised to these organelles may functionally cooperate to control cell migration or cell size. There is however evidence that surface membrane potential and plasma membrane tension can influence mTORC1 (and mTORC2). Indeed, hyperpolarisation of the membrane can restore ectopic mTORC1 activity in a model of senescence[39] while decreased tension leads to a rearrangement of PIP2 and inactivation of mTORC2[81].

mTORC1 beyond the lysosome

It is an attractive, logical model whereby amino acids promote the recruitment of mTORC1 to Rheb-enriched lysosomes, and subsequently re-localise these mTORC1-positive lysosomes to the cell periphery where Rheb-GTP loading is enhanced by activated growth factor receptor/ PI3K/Akt signalling. In the next sections, we will explore how this model spatially integrates with our expanding understanding of how the endosomal, autophagosomal and secretory pathways transduce nutrient availability and control mTORC1 localisation and activity.

2.6 mTORC1 and the endosomal pathway

The endosomal pathway controls the internalisation and turnover of surface proteins such as receptors, adhesion molecules and transporters which are taken up into Rab5-decorated early endosomes and then either trafficked back to the cell surface or targeted for degradation via late endosomes and lysosomes[82]. Several studies indicate the endosomal pathway can impact mTORC1 activity (and vice versa) via a number of both direct and indirect mechanisms, including via delivery of nutrient receptors to the cell surface[83], controlling protein topography on the lysosomal membrane [84]and delivery of RagD- and SLC38A9-positive endosomes to endolysosome compartments[85] (**Figure 1**).

The endosomal pathway has been implicated in regulating both growth factor-dependent and Rag-dependent activation of mTORC1 [43, 45, 83-85]. For example, genetic inhibition of dynamin-dependent endocytosis [45] and depletion of components of the major endosomal recycling complex, retromer [83, 84], reduces mTORC1 activity. Retromer loss leads to gross changes in the localisation of proteins on the surface of the lysosome. TBC1D5, a Rab7 GAP and retromer-interacting protein can restrict the localisation of Rab7a to discrete regions on the lysosomal membrane. The loss of TBC1D5 leads to a redistribution of Rab7a and significant increase in Rab7 co-localisation with LAMTOR, proteins which are usually spatially segregated[84]. The authors propose this leads to reduced Rag GTPase and mTORC1 localisation to the lysosome, changes in lysosomal distribution, and decreased mTORC1

activity. At the same time, retromer is also required for the adaptive delivery of glutamine transporters such as SNAT1 (SLC38A1) and LAT1 (SLC7A5) to the cell surface during starvation to facilitate nutrient uptake[83] (**Figure 1**). Several endosomal regulators [85] and retromer subunits have been identified as transcriptional targets of TFEB and are thus upregulated upon starvation to promote the roles described above (**Figure 2**).

Proper maturation through the endosomal pathway controls nutrient-dependent mTORC1 activity. TFEB-regulated endocytosis supports the generation of so-called 'signalling endosomes' that are required to deliver mTORC1-associated machinery to late endosomes and activate Akt [85]. At the same time, while Rab5-dependent PI3P production, downstream of amino acids normally promotes the activation of mTORC1 [86], overexpression of constitutively active Rab5 ($Rab5^{Q79L}$) leads to the formation of distinctive, enlarged hybrid structures as a result of impaired transition of early-to-late endosomes, and inhibits mTORC1 in response to both amino acids and growth factors[87]. Interestingly, only overexpression of Rheb, not activation of endogenous Rheb (i.e. TSC2 knock-out) can activate mTORC1 in these cells. Coupled with the fact that the mTORC1 complex localises to the Rab5-positive hybrid endosomes, together, these data support a model whereby mTORC1 is spatially segregated from Rheb in Rab5 over-expressing cells, supporting the hypothesis that Rheb is localised to the mature late endosome and/or lysosome. These data are interesting considering the new studies discussed in section 2.3, given that Rheb could presumably still transiently interact with the membrane of these Rab5-positive, mTOR-positive structures. It is possible that in cells with defective endosomal maturation, the large, dynamic mTORC1-regulating complex is not properly assembled.

The spatial control of lipids is essential for the proper regulation of endosomal and other membrane trafficking pathways and can impact mTORC1 via multiple mechanisms. The class III PI3K, hVps34 represents an interesting spatial regulator of the mTORC1-autophagy pathway by forming multiple complexes that act both upstream and downstream of mTORC1 to control the activation of both mTORC1 and autophagy. Vps34 is a well-known regulator of autophagy, and forms a complex with Beclin1, Atg14 and Vps15 to promote PI3P required for autophagosome formation, and indeed mTORC1 phosphorylation of Atg14 in this complex inhibits the induction of autophagy [88]. Furthermore, mTORC1 phosphorylation of UVRAG in another Vps34-containing complex controls PI3P-dependent tubulation of autolysosomal membranes to support the resolution endo/autolysosomes [89]. At the same however, Vps34 - dependent production of PI3P in response to amino acids and glucose has also been shown to act upstream of mTORC1 and be a positive regulator of its activity. Production of another lipid, $PI(3,4)P_{2,}$ produced in the vicinity of RPTR at the lysosome by class II PI3K b (PI3K C2b) represses mTORC1 by promoting association between 14-3-3 and RPTR. Thus, lipid kinases

in complex with different regulators participate in the tight spatial control of lipid synthesis to control the balance between mTORC1 and autophagy activity.

Our understanding of the complex, direct and indirect ways in which the endosomal pathway influences nutrient-dependent mTORC1 is growing rapidly. The simplistic view that endocytosis and recycling control mTORC1 through localisation of surface receptors has expanded to include the specific delivery of regulatory proteins, complexes and lipids to ensure tight spatial and temporal control of mTORC1 signalling. At the same time, we are only just beginning to understand how different nutrient uptake routes i.e. transporters vs endocytosis vs macropinocytosis integrate and cooperate with lysosome-derived amino acids and growth factor signalling to control the balance between cellular anabolism, catabolism and survival. Indeed, macropinocytotic uptake and utilisation of extracellular protein as a nutrient source to activate mTORC1 only occurs in amino acid-depleted conditions. In these low amino acid, high protein culturing conditions, active mTORC1 actually inhibits proliferation, which together indicates mTORC1 can not only sense the quality and source of nutrients but that that cells significantly favour the uptake and use of free amino acids [46, 47]. At the moment it is not clear where or how this sensing occurs, and future work will expand our knowledge of how nutrients are taken up, sensed and utilised via the mTORC1 pathway.

2.7 Spatial association of mTORC1 and autophagy

As mentioned previously, there is tight spatial cycling between the activity of mTORC1 and autophagy that is essential to control cell growth and survival via balancing anabolic versus catabolic metabolism[4]. In particular, the ER is a central regulator of the tight spatial association between lysosome function, mTORC1 activity and autophagy. The close association of mTORC1-positive lysosomes with ER also spatially coordinates with autophagy initiation which is negatively controlled by mTORC1-dependent phosphorylation of ULK1, the autophagy initiating protein[4, 5]. Inhibition of mTORC1 leads to the recruitment of ULK1 complex to the ER membrane, which together with Vps34-containing class III PI3K complex and Atg9-decorated vesicles derived from the Golgi participates in the formation of the PI3P-enriched omegasome. By sequestering membrane from various intracellular sites including ER, plasma membrane and Golgi [5, 90], sites also implicated in controlling mTORC1 activity [91], the omegasome develops into immature phagophore and from there, a mature autophagosome. En-route to the lysosome, autophagosomes can interact with the endosomal pathway to form hybrid structures called amphisomes. Ultimately, cargo from starvation-induced autophagosomes and amphisomes is delivered to and degraded by lysosomes and can then directly reactivate mTORC1 (**Figure 2**).

Another key regulator of the complex interplay between mTORC1 and autophagy are members of the TFEB-family of transcription factors. Starvation-induced translocation of TFEB to the nucleus can ultimately impact mTORC1 activity both directly and indirectly by controlling the expression of RagD and autophagy/lysosomes genes, respectively [6, 92].

mTORC1 and the secretory pathway

In the next section we will discuss the increasingly evident role for the ER and Golgi in controlling mTORC1, from signalling, to membrane tethering and membrane-membrane contact sites.

2.8 mTORC1 and Golgi

Several studies have observed mTORC1 localising to the Golgi membrane independently and in addition to the pool of mTORC1 on lysosomes [93-95]. At the same time, the Golgi-associated small GTPases Arf1 and Rab1 can activate mTORC1 via poorly understood mechanisms [43] (**Figure 2**). Arf1 specifically transduces glutamine availability to mTORC1 via a mechanism that promotes mTORC1 to the lysosomal membrane independently of Rag-Ragulator but still dependent on V-ATPase[17]. Further studies implicate the Golgi-resident amino acid transporter, PAT4 in recruiting mTORC1 to Golgi membranes which is important to sense the availability of glutamine and serine in fast growing colorectal cancer cells[94]. Similarly, GTP loading of Rab1 by amino acids can activate mTORC1 and in cancers such as colon cancer, Rab1 overexpression and increased mTORC1 drive tumorigenesis [96]. There is certainly an emerging picture whereby the Golgi is important for sensing glutamine availability to mTORC1 and it remains to be seen to which extent this is mediated by signal transduction or direct interactions. As a conditionally essential amino acid, glutamine is particularly important in fast-growing cells such as cancer cells and thus cells with different metabolic demands may utilise different or additional pools of mTORC1 to sustain increased growth and proliferation.

2.9 mTORC1 and ER

The endoplasmic reticulum (ER) is a site that coordinates the folding and transport of new proteins, as well as sorting and recycling of proteins via the endosomal pathway. There are no reports that mTORC1 localises to the ER but there are both direct and indirect mechanisms via which ER can influence mTORC1 activity. In particular, that the master activator of mTORC1, Rheb has been widely observed on ER membranes and previous reports have suggested that contact sites between lysosome-bound mTORC1 and ER membranes facilitate mTORC1-Rheb interactions (as discussed above).

A number of ER-resident proteins can indirectly influence mTORC1 activity by controlling the localisation of lysosomes, including protrudin- (via Rab7-FYCO1-kinesins)[59, 65] and VAPA- (via the lysosomal cholesterol sensor, ORP1L and Rab7) in response to nutrient availability.

Further to this, recent evidence indicates that lysosome-ER contact sites that facilitate the flux of cholesterol can directly influence mTORC1. Indeed, apart from amino acids, cholesterol is only other metabolite currently known to activate mTORC1 from within the lysosome, and it does so via a Rag-dependent mechanism involving the cholesterol transporter NPC1 and SCL38A9 but independent of amino acid availability [97] (**Figure 2**). Mutations in NPC1 cause the neurodegenerative, lysosomal storage disease Niemann-Pick disease type C1 which is characterised by the accumulation of lysosomal cholesterol, impaired autophagy and ectopic activation of mTORC1[97-99]. A recent study demonstrated that these phenotypes are caused by the VAPA-ORP1L-dependent, ER-to-lysosome transfer of cholesterol which fails to be effluxed efficiently by mutant NPC1. Inhibiting cholesterol transfer to the lysosome can restore mTORC1 and autophagy in NPC1-null cells [99] while activation of autophagy can also help in clearing cholesterol-filled lysosomes [98] and as discussed above, the ER represents an important co-ordinator for the mTORC1-autophagy pathway.

2.10 mTORC1 in the nucleus

Numerous studies have identified mTOR in the nucleus, both as a part of mTORC1 and mTORC2 [100]. Inhibition of nuclear export by leptomycin B leads to an accumulation of mTOR and Raptor in the nucleus [101, 102], and inhibition of pS6K and p4EBP1 [102] suggesting that nucleo-cytoplasmic shuttling of mTORC1 occurs. Most reports however agree that mTORC1-dependent phosphorylation of downstream targets occurs primarily in the cytoplasm [103, 104]. There are however reports that pools of mTORC1 in the nucleus may contribute to transcriptional regulation in health [105] and disease [106]. Furthermore, independent work from two labs indicates that phosphorylation of TFEB by mTORC1 is required for its exit from the nucleus [107, 108] and although it has not yet been formally addressed, could it be possible that nuclear mTORC1 phosphorylates TFEB?

2.11 mTORC1 in the cytoplasm

A third, cytoplasmic mTOR complex 3 was recently described whereby mTOR associates with the transcription factor ETV7, independently of its function as a transcription factor, and forms a rapamycin-insensitive complex. ETV7 is highly upregulated in a wide range of cancers, including 85% of medulloblastoma's and the authors propose that rapamycin insensitivity observed in many cancers is a result of increased mTORC3 [109].

3. Conclusion

Complex mechanisms have evolved to maintain optimal levels of cell growth in response to constantly changing nutrient availability. At the heart of this lies the mTORC1 signalling network which, as we have discussed is controlled by tight, nutrient-responsive changes in subcellular localisation of multiple regulators. The nutrient-dependent cytoplasm-lysosome

shuttling of mTORC1 components coupled with the dynamic movement and interaction of mTORC1-decorated lysosomes between distinct regions of the cell including plasma membrane, ER and Golgi allow for exquisite control of mTORC1 activation. Moving ahead, the field will undoubtedly reveal new insights into the spatial control of mTORC1; just a few of the important questions remaining include how and where the Rheb-mTORC1 interaction is controlled, to what extent do different sources of nutrients (i.e. autophagy vs macropinocytosis vs nutrient transporters) effect mTORC1 activity, how are amino acid signals transduced from the Golgi to the lysosome and a better mechanistic understanding of how maturation through the endosomal pathway controls mTORC1.

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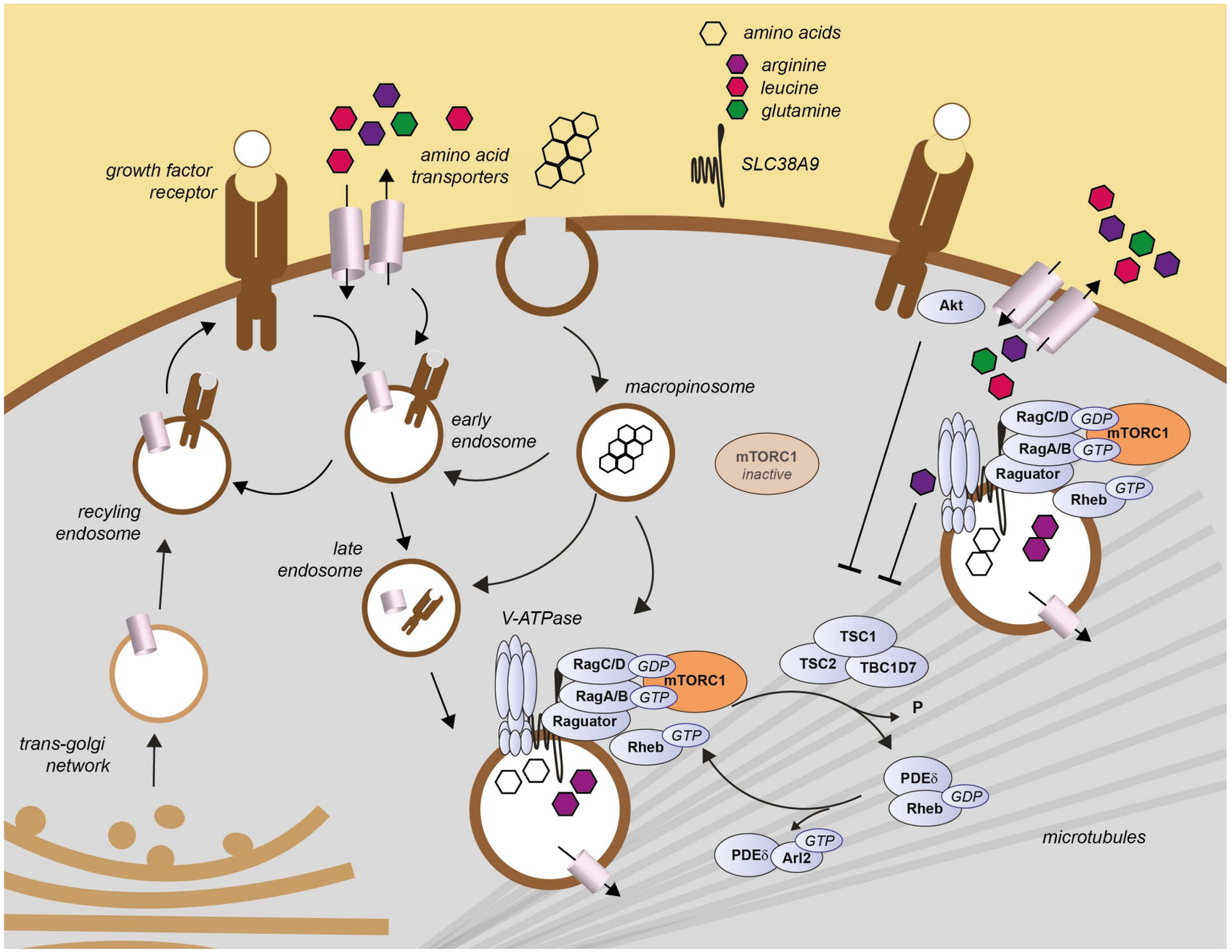

amino acids
arginine
leucine
glutamine
SLC38A9
growth factor receptor
amino acid transporters
macropinosome
early endosome
recyling endosome
late endosome
trans-golgi network
V-ATPase
mTORC1 inactive
Akt
RagC/D
GDP
mTORC1
RagA/B
GTP
Raguator
Rheb
GTP
TSC1
TSC2
TBC1D7
P
PDEδ
Rheb
GDP
PDEδ
Arl2
GTP
microtubules

Figure 1: mTORC1 and the lysosome

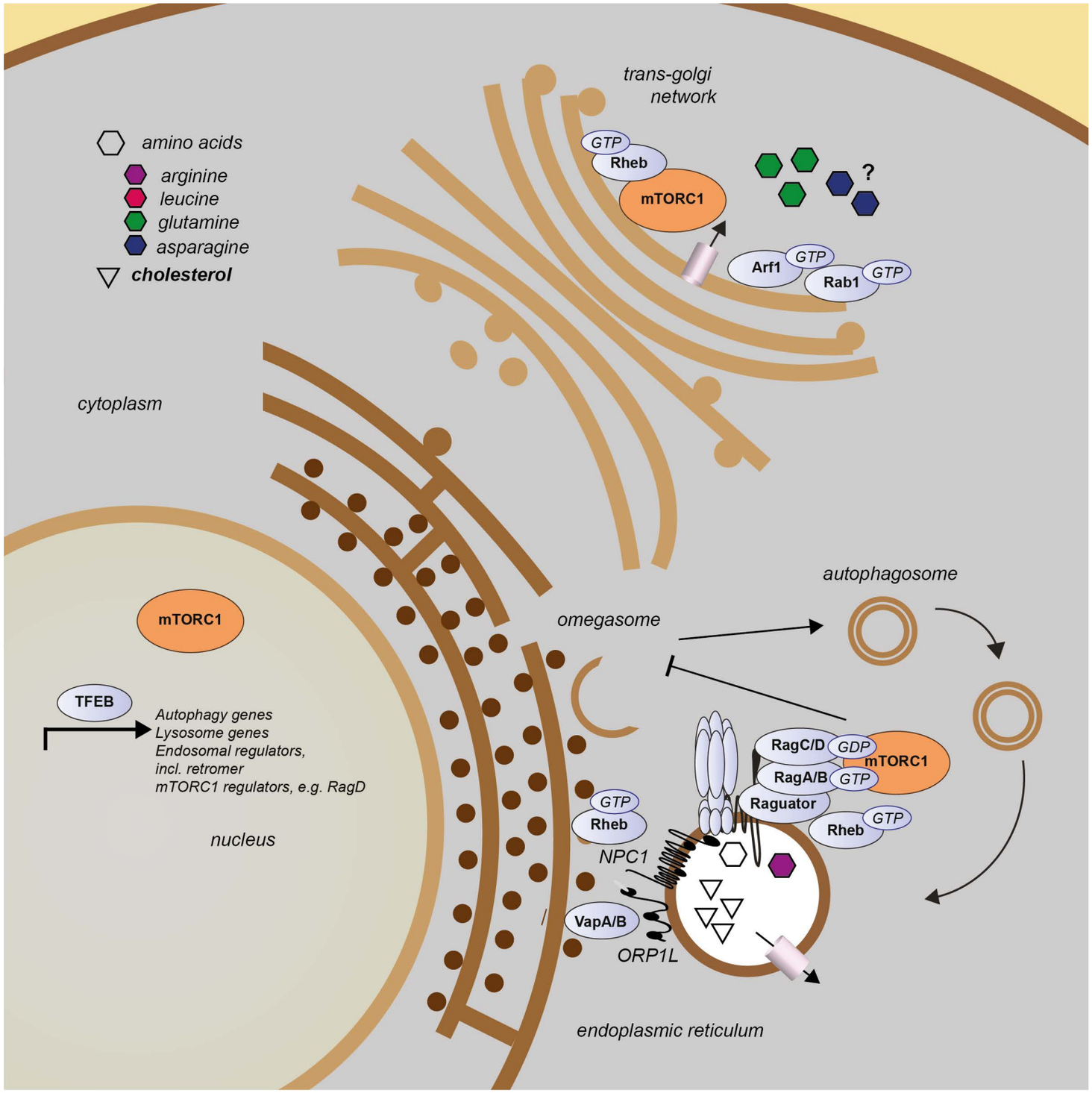
mTORC1 has been observed at various sites in the cell including the nucleus, cytoplasm and Golgi but it's main site of activation is on the lysosome (also see Figure 2). mTORC1 is recruited to the lysosome via a complex including Rag GTPases, Ragulator, V-ATPase and SLC38A9 among others. The nutrient-dependent re-localisation of mTORC1-positive lysosomes, along microtubules to the cell periphery may support mTORC1 activity by bringing it into close proximity with amino acids and growth factor signals at the plasma membrane. Starvation leads to the re-distribution of lysosomes to the juxtanuclear region where they are tethered to ER and Golgi membranes (see Figure 2). Clustering of lysosomes correlates with decreased mTORC1 and increased autophagy.

The localisation of mTORC1 to the lysosomal membrane is dependent on the availability of amino acids which can derive from the extracellular environment or intracellular sources such as lysosomal degradation. Amino acid sensing has been noted in the cytoplasm, in the lysosome lumen and via poorly understood mechanisms via the Golgi (Figure 2). Recruitment of mTORC1 to the lysosome is widely considered to facilitate the direct interaction of mTORC1 with its activating small GTPase, Rheb. Rheb-GTP status is controlled downstream of growth factors and amino acids by the Rheb GAP, TSC complex (TSC1, TSC2 and TBC1D7). The localisation of Rheb is still a poorly understood area of mTORC1 signalling, with reports placing the small GTPase at multiple sites including ER, Golgi and peroxisomes in addition to the lysosome (see Figure 2). Recent evidence suggests that Rheb localisation to the lysosome may be controlled via nucleotide-dependent interaction with PDEδ, a solubilising factor or via transient membrane interactions.

The transcription factor, TFEB is an important regulator of the spatial control of mTORC1 via expression of mTORC1 regulators (e.g. RagD), autophagy and lysosomal genes and endosomal regulators such as components of the retromer complex, which facilitates the adaptive delivery of amino acid transporters from the Golgi to the cell surface in starvation.

Figure 2: mTORC1 beyond the lysosome

As well as the lysosomal surface, the cytoplasm, Golgi and nucleus have all been implicated in the regulation or activation of mTORC1. The Golgi has been particularly implicated in the transduction of amino acids such as glutamine via the small GTPase Arf1. More recently, Arf1 was also shown to be important for transducing the availability of asparagine while another Golgi localised small GTPase, Rab1a is further implicated in mTORC1 activation. Lysosomal-ER contact sites have been implicated in controlling mTORC1 activity via direct and indirect mechanisms, including the sensing of cholesterol, spatial association with autophagy and as the primary site for Rheb localisation.


amino acids
arginine
leucine
glutamine
asparagine
cholesterol
trans-golgi network
GTP
Rheb
mTORC1
?
Arf1
GTP
Rab1
GTP
cytoplasm
autophagosome
omegasome
mTORC1
TFEB
Autophagy genes
Lysosome genes
Endosomal regulators, incl. retromer
mTORC1 regulators, e.g. RagD
nucleus
RagC/D
GDP
RagA/B
GTP
mTORC1
Raguator
GTP
Rheb
GTP
Rheb
NPC1
VapA/B
ORP1L
endoplasmic reticulum